clumps. The resulting crude virus stock is then inoculated in serial 10-fold dilutions onto 143 cells (Mackett, M., et al, *J Virol* (1984) 49: 857–864), a human tk- cell line. After two hours at 37° C., the 143 monolayers are then overlaid with 1% agarose containing 1× modified Eagle's medium, 5% fetal bovine serum, and 25 μg/ml 5-bromodeoxyuridine (BUdR). After incubation for two days at 37° C., BUdR-resistant plaques are picked and grown for 48 hours at 37° C. in 24 well plates of 143 cell monolayers in the presence of 25 μg/ml BUdR.

Virus is harvested and assayed for the presence of the EIV-derived gene by DNA-DNA dot blot hybridization, as follows: cells are scraped from the dish into an Eppendorf centrifuge tube, centrifuged for 1 minute, and the cell pellet resuspended in 0.2 ml PBS. After freeze-thawing 3 times and sonicating as described above, the sonicate is applied to a nitrocellulose filter and air dried. A wild-type virus control is also spotted onto the same filter. The filter is then placed on paper soaked in (1) 0.5M NaOH, (2) 1M Tris-HCl, pH 7.5, and (3) 2× SSC, for 5 minutes each. The filter is then baked at 80° C. under vacuum for 2 hours. The baked filter is prehybridized for 1–4 hours at 42° C. in 5 ml 50% formamide, 4× SSC, 5× Denhardt's solution, and 0.1 mg/ml sheared and boiled salmon sperm DNA. The filter is then hybridized overnight at 42° C. to 2×10^7 cpm of ^{32}P-labeled EIV probe in 5 ml prehybridization buffer. The probe is prepared by isolating 5 μg of EIV gene insert from one of the pGS20-derivative vectors and nick translating the DNA using a commercially available nick translation kit. The filter is then washed twice for 30 minutes in 0.5× SSC, 0.1% SDS, air dried, and autoradiographed overnight at −70° C.

Virus containing EIV gene inserts by dot blot hybridization are infected onto monolayers of 143 cells and left until a confluent cytopathic effect is obtained. The culture medium is then aspirated off and the cells lysed in 1% SDS, 0.1M β-mercaptoethanol, 50 mM Tris-HCl, pH 7.8. The lysate is made 0.5 mg/ml in proteinase K, incubated 4 hours at 37° C., phenol extracted, ethanol precipitated, and analyzed by restriction enzyme analysis to show that the vaccinia genomes indeed contain the desired EIV genes.

The recombinant vaccinia so generated were designated vac-H7, vac-H3, vac-N7 and vac-N8 or collectively, vac-EIV.

D.5 Expression of EIV Surface Antigens by Vaccinia Recombinants

Each vac-EIV recombinant was plaqued separately onto CV1 monolayers. The monolayers were washed, fixed, and incubated with rabbit anti-EIV-A1 (for vac-H7 and vac-N7) and rabbit anti-EIV-A2 (for vac-H3 and vac-N8). Antibody binding specificially to EIV surface antigens was detected by a subsequent incubation with ^{125}I-labeled staphylococcal A protein followed by autoradiography. In each case, all recombinant virus plaques bound the appropriate antibody.

Immunoprecipitation of ^{35}S-labeled cellular lysates obtained from monolayers of CV1 cells infected by individual vac-EIV recombinants showed the presence of EIV protein. In the case of each recombinant virus, antisera raised against the appropriate whole virus immunoprecipitated a characteristic hemagglutinin or neuraminidase molecule identified by polyacrylamide gel electrophoresis and autoradiography.

D.6 Amplification of Vaccinia Recombinants

After the vac-EIV recombinants were identified as both carrying the appropriate EIV gene and expressing the encoded EIV protein, a single plaque of each is isolated ×2 in 143 cells in the presence of BUdR. The twice purified isolate is amplified once in 143 cells in the presence of BUdR and subsequently in CV-1 cells in the absence of selective pressure. The resulting vac-EIV recombinants are stable genetically and can be further amplified in CV-1 cells.

D.7 Biossay of Vaccinia Recombinants

Horses are used as subjects to assess the ability of the recombinant vaccinia to raise titers of neutralizing anti-EIV antibodies in serum.

In general, pairs of horses are inoculated with wild-type or recombinant virus by intradermal administration of $1\text{–}2\times 10^8$ plaque-forming units distributed in 2–3 sites on the back, and are bled at days 0, 14, and 28. The sera are then tested for the presence of anti-EIV neutralizing antibodies in the plaque reduction assay of ¶C.7.

In one experiment, four horses, 1–3 years old, housed in a P3 facility at Washington State University, Pullman, Wash., were ascertained to be serum negative with respect to anti-EIV antibodies, but were otherwise undocumented with respect to exposure. Control and test virus were administered intradermally by scarification with a needle to bleeding at four locations on the animals' necks. Each animal received a total of 10^8 pfu of each indicated virus in a total of 0.4 ml (0.1 ml at each scar). VSV was used as a control; test viruses were H7, H3, N7, and N8, as described above ¶D.4).

The animals were bled at day 0 (preimmune), and at days 7, 14, and 21, and their sera assessed for neutralizing antibodies against each of EIV-A1 and EIV-A2 using the plaque reduction assay described in ¶C.7 hereinabove. The results, shown below in Table 7, indicate that while control VSV was not able to raise antiserum against EIV, all combinations of recombinant vaccinia containing DNA encoding H and/or N peptides, were able to do so.

TABLE 7

Horse	Vaccine	EIV Strain	Day 0	7	14	21
Lola	VSV	A1	1/10	1/10	1/10	1/10
		A2	<1/10	<1/10	<1/10	<1/10
Howard	N7,N8	A1	1/10	1/20	1/160	1/160
		A2	<1/10	1/40	1/160	1/160
Fanny	H7,H3	A1	<1/10	1/160	1/320	1/160
	N7,N8	A2	<1/10	1/320	1/640	1/320
Leo	H7,H3	A1	<1/10	1/800	1/1600	1/800
		A2	<1/10	1/640	1/1280	1/640

The results are maximum serum dilutionss able to reduce EIV pfu by 50%. The results for Leo at days 7, 14, and 21 are based on an initial serum dilution of 1/50 rather than the 1/10 dilution used for all other results.

We claim:

1. A recombinant DNA sequence encoding equine hemagglutinin H7 as depicted in FIG. 1.

2. A recombinant DNA sequence encoding equine neuraminidase N7 as depicted in FIG. 3.

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